Toluidine Blue – Basic Fuchsin for Plastic Sections (Thin) - DRAFT

Prep: Cut at 5µm.

Safety: **DANGER!** Ethanol is highly flammable and can cause serious eye irritation and organ toxicity. Formic acid is a flammable liquid and vapor. Causes severe skin burns and eye damage. Toxic if inhaled.

WARNING! Basic fuchsin is harmful if swallowed and is suspected of causing cancer.

Solution A: 1% Toluidine blue

1g Toluidine blue (Tol blue C.I. #52040; CAS #92-31-9)
1g sodium (tetra) borate
100mL dH20
Mix well. Filter through gauze, if desired. Stable for at least 6 months at room temperature.

Stock solution B: Basic Fuchsin

1g Basic fuchsin powder (CI #42510)
100mL of 50% ethanol
Mix well. Stable for up to 6 months at room temperature.

Working Solution A – 0.21% Basic Fuchsin

3mL of stock solution
60mL dH20
Mix well, no need to filter, discard after use.

Equipment: Oven (or water bath) set to 37°C to warm the Toluidine blue.

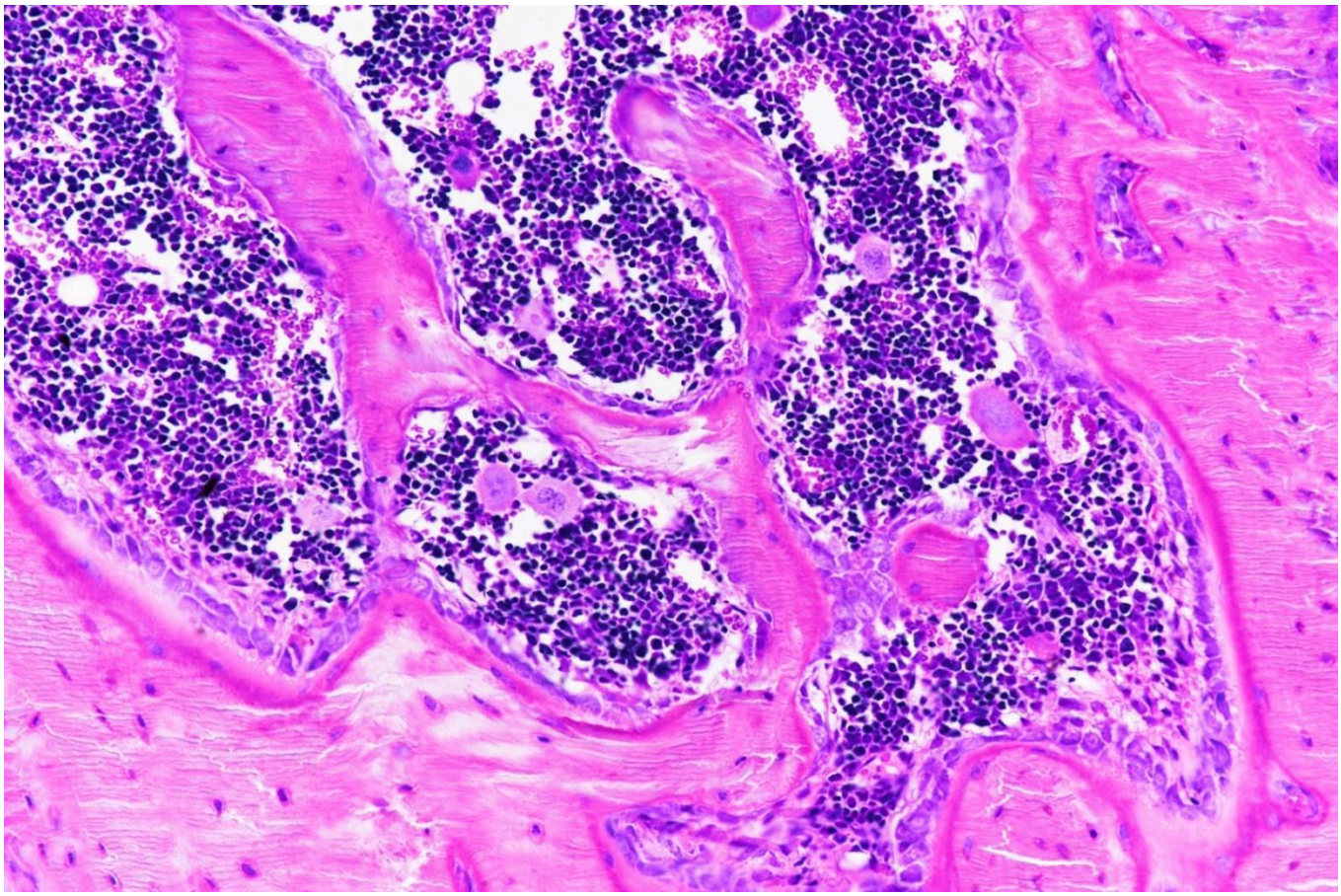
Protocol

1. Deplasticize in acetone 3 x 20 min each and hydrate to dH20.
2. Place warmed Toluidine blue solution on each slide for 30 seconds - 5 min (or stain using a staining dish). Optimization of controls is necessary!
3. dH2O – quick rinse
4. 70% EtOH – 30 seconds to 1 min.
5. Basic fuchsin working solution – 10 sec to 1 min (30 seconds average). NOTE: stain will fade a little during dehydration.
6. dH2O – quick dip.
7. 70% EtOH – 30 seconds to 1 min.
8. 95% ethanol - 30 seconds to 1 min.
9. 100% ethanol - 30 seconds to 1 min.
10. If stain is still too dark, destaining may be done for several more minutes in water or quickly in 70% ethanol as needed, followed by a second dehydration.
11. Clear and mount using a solvent-based mounting medium.

Timing for Tol blue and BF steps may vary with the type of tissue or plastic section.

Results:

Calcified bone	Pink
Young bone	Dark pink
Osteoid	Bluish purple
Soft tissue/cell cytoplasm	Light blue or purple
Nuclei	Blue



Mouse femur, 200x, 30 seconds in each stain.

References: Abreu, Monica et al: Toluidine Blue-Basic Fuchsin Stain for Glycol Methacrylate Embedded Tissue. J of Histotechnology / Vol. 16, No. 2 / June 1993

Osborne, Daniel L. and Curtis, Janene, "A Protocol for the Staining of Cement Lines in Adult Human Bone Using Toluidine Blue" (2005)